REMARKS

Claims 10, 13-15, and 39-68 are are pending, claims 1-9, 11, 12, and 16-38 having been canceled by the present amendment. New claims 39-68 have been added.

The amendment to claim 10 is supported by disclosure on page 4, lines 1-4, and on page 13, line 10, of the specification. New independent claims 43, 51, and 59 are supported by disclosure on page 4, lines 1-4, of the specification. New claims 39, 47, 55, and 63, are supported by disclosure on page 3, line 23, of the specification. New claims 40, 48, 56, and 64, are supported by disclosure on page 17, line 29, of the specification. New claims 41, 49, 57, and 65 are supported by disclosure on page 17, line 29, of the specification. New claims 42, 50, 58, and 66 are supported by disclosure on page 17, line 28 of the specification. New claims 44, 52, and 60 are supported by disclosure on page 1, line 26, of the specification. New claims 45, 53, and 61 are supported by disclosure on page 1, lines 25-29, of the specification. New claims 46, 54, and 62 are supported by disclosure on page 1, line 30, of the specification. New claim 67 is supported by disclosure on page 47, lines 30-31, of the specification. New claim 68 is supported by disclosure throughout the specification, e.g., at page 47, line 26, to page 48, line 26.

No new matter has been added by this amendment.

35 U.S.C. § 102(e)

Claims 10, 11, 13, and 14 were rejected for anticipation by Radosevich et al. On page 6, lines 6-10, of Paper No. 10, the Examiner states:

> Radosevich discloses that the protein coding region of the labyrinthin gene comprises the protein coding region of the HAAH gene (column 7, lines 7-11). Radosevich discloses the use of the full-length antisense labyrinthin cDNA to reduce the growth rate of A549 cells (column 9, second paragraph), which are tumor cells derived from a hepatocarcinoma.

Claim 11 has been canceled. Claim 10 has been amended to require that the HAAH inhibitory compound is a HAAH antisense nucleic acid, which is complementary to a 5' HAAH regulatory region. Although HAAH and labyrinthin ("lab") share some sequence identity in the internal regions of the proteins, the 5' regulatory (e.g., untranslated) region, and 5' coding region (e.g., sequences encode by sequences in exon 1 or sequences encoding a 5' signal peptide) are different. The differences between HAAH and lab are described in Radosevich at col. 7, lines 7-11, to which the Examiner refers:

> Although the protein coding region of lab is identical to an internal region of the sequence reported for HAAH, the 5' untranslated region of HAAH is different, and part of the 5' translated protein coding region of HAAH is missing from that found in the lab clone.

Thus, the antisense nucleic acids required by the amended claims are in regions in which HAAH and lab are different. Thus, claims 10, 13, and 14 are not anticipated by Radosevich.

Moreover, contrary to the Examiner's statement, A549 cells are derived from a human lung cancer, i.e., an adenocarcinoma, rather than a hepatocarcinoma. Thus, claim 14 is further distinguished from the Radosevich et al.

Radosevich fails to describe methods using the HAAH antisense sequences required by the amended claims and fails to describe treating the types of cancers required by the claims. Thus, this rejection should be withdrawn.

35 U.S.C. § 112, first paragraph

Claims 10-15 were rejected for overbreadth and lack of enablement. On page 2, lines 10-15 and 21-24, of Paper No. 10, the Examiner stated:

> while being enabling for a method for inhibiting growth of a mammalian tumor cell in culture or to a method for inhibiting a mammalian tumor cell line grown in culture, said methods comprising the administration of a HAAH antisense nucleic acid consisting of the full length antisense HAAH cDNA as well as antisense

> DNA corresponding to exon 1 of the HAAH gene, does not reasonably provide enablement for a method for inhibiting tumor growth in a mammal comprising the administration of a HAAH antisense nuclei acid, ribozyme, or intrabody....Demonstrating the inhibition of aspartyl beta hydroxylase expression in tumor cells cannot alone support the predictability of the method for prevention of or treating said tumor growth through administration of either an antisense nucleic acid or an intrabody directed to aspartyl beta hydroxylase.

The claims have been amended to require that the antisense nucleic acids used in the claimed methods correspond to sequences in defined regions of the AAH gene, e.g., 5' AAH regulatory region, 5' AAH protein coding region, AAH sequence encoding a signal peptide, AAH sequences in exon 1 of the AAH gene, or sequences corresponding to the full-length naturally-occurring AAH cDNA. Claims drawn to administration of a ribozyme or intrabody have been deleted.

With respect to claims drawn to methods of inhibiting tumor growth using AAH antisense nucleic acids, Applicants submit that the specification coupled with the knowledge in the art of molecular biology and antisense technology fulfills the requirements of §112.

The enablement standard requires that the specification provide a description that, when coupled with the knowledge possessed by a person of ordinary skill in the art, enables that person to make and use the claimed invention.[1] Enablement is not precluded by the necessity for some experimentation; however, any required experimentation must not be undue experimentation.[2] "The key word is 'undue,' not 'experimentation.'"[3]

Regarding claim scope, the factors to be analyzed in determining whether undue experimentation is required to practice the full scope of the claims are discussed in In re Wands.[4]

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[1] Atlas Powder Co. v. E.I. duPont De Nemours & Co., 750 F.2d 1569, 1576 (Fed. Cir. 1984).
[2] In re Wands, 858 F. 2d 731, 736-7 (Fed. Cir. 1988).
[3] In re Angstadt, 537 F. 2d 498, 504 (C.C.P.A. 1976).
[4] In re Wands, 858 F.2d 731, 736-7 (Fed. Cir. 1988).

Undue experimentation would not be required to practice the claimed methods. The court in In re Wands set forth eight factors to be considered in determining whether undue experimentation would be required: (1) the quantity of experimentation necessary, (2) the amount of direction or guidance presented, (3) the presence or absence of working examples, (4) the nature of the invention, (5) the state of the prior art, (6) the relative skill of those in the prior art, (7) the predictability or unpredictability of the art, and (8) the breadth of the claims.

Quantity of Experimentation Necessary

In Wands, the Court stated,

> [A] considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed.

Applying this criterion here, all of the techniques required to practice the claimed methods were described in the specification or well-known to those skilled in the art as of the filing date.

As is discussed below, little or no experimentation is required to determine which antisense nucleic acids to use to inhibit tumor cell growth. The nucleotide sequence of AAH, e.g., HAAH, is known in the art. Armed with the AAH sequence, the specific sequence limitations which are now recited by the claims (and described in the specification) are unambiguous. Little or no experimentation would be required by the skilled artisan to determine the sequence of an antisense nucleic acid, which is complementary to a 5' AAH regulatory

sequence, 5'AAH coding region, signal peptide, exon 1 sequence, or entire AAH cDNA sequence.

The amount of experimentation required to administer an antisense nucleic acid is also minimal. Clinical methods of administering an antisense therapeutic agent are well established and described in the specification, *e.g.,* intravenously, subcutaneous, intramuscularly, intraperitoneally, or directly into affected tissue (page 19, lines 15-24, of the specification).

Given the teachings of the specification, the amount of experimentation required to determine what antisense nucleic acid to administer and how to administer it to a mammal for the purpose of inhibiting tumor cell growth is not undue.

Amount of direction or guidance presented

The greater the amount of guidance provided, the more this factor weights in favor of granting the claim. The specification of the present application provides ample guidance regarding the procedures required to carry out the methods of the invention.

The specification provides extensive description of the AAH antisense nucleic acids to be used for inhibiting growth of tumor cells. For example, the specification (at page 4, lines 1-4) teaches that the antisense nucleic acid is complementary to the 5' AAH regulatory sequence, the 5' portion of the coding sequence of AAH mRNA, a sequence encoding a AAH signal peptide, a sequence within exon 1 of the AAH gene, or the entire full-length naturally-occurring AAH cDNA (page 47, line 30-31, of the specification). Full-length and shorter (e.g., 10-50 or 10-20 nucleotides in length are disclosed on page 4, lines 10-14, of the specification, and methods of making the antisense molecules is taught on page 47, line 30, to page 48, line2, of the specifiction. Moreover, methods of testing AAH antisense nucleic acids for their ability to decrease production of AAH in

tumor cells is described on page 18, lines16-24, of the specification and the results of such assays is described in Example 5 (page 47, line 26, to page 48, line 26, of the specification). Thus, little or no experimentation is required to identify which AAH antisense nucleic acids are to be administered according to the amended claims.

The specification is equally exhaustive in describing how to carry out methods for inhibiting growth of tumor cells. Formulations such as vectors and gene delivery systems are described on page 18, line 29, to page 19, line 14, of the specification. Routes of administration and dosages are described on page 19, lines 15-32, of the specification.

With respect to inhibition of central nervous system (CNS) tumors, the Examiner raises the issue of difficulties associated with the blood-brain barrier, stating:

> it is well known in the art that the use of modified anti-sense oligonucleotides on CNS targets are limited by the powerful ability of the blood brain barrier to exclude such anti-sense oligonucleotide (page 3, lines 22-24, of Paper No. 10).

Although an intact blood brain barrier may pose a drug delivery problem, in the case of CNS tumors, compromise of the blood-brain barrier allows systemically delivered drugs to pass through the barrier into the CNS (page 19, lines 20-22, of the specification. Other approaches to delivering drugs through the blood brain barrier, including liposomal antisense compositions, and direct infusion into cerebrospinal fluid, are described on page 19, line 19, and lines 22-24, of the specification. Moreover, Broaddus (cited as being indicative of the state of the art) indicates that numerous promising approaches to drug delivery across the blood brain barrier are known (e.g., direct cerebrospinal infusion or positive pressure infusion techniques; page 135 of Broaddus)

The teachings provided in the specification are sufficient to enable one skilled in the art of antisense technology, *e.g.*, a molecular biologist, to make the antisense nucleic acids required

by the amended claims, and for one skilled in the medical arts, e.g., a physician, to administer such nucleic acids to cancer patients.

Presence or absence of working examples

The lack of an *in vivo* working example appears to be at the heart of the Examiner's reasons for rejecting the claims for lack of enablement. The Examiner states:

> The specification teaches the use of the full length antisense HAAH cDNA as well as antisense DNA corresponding to exon 1 of the HAAH gene were used to decrease the level of expression of the HAAH polypeptide in heptatocyte carcinoma cells, and alter the morphology of the treated cells to resemble a more differentiated phenotype. The specification does not teach the decreased level of expression of the HAAH polypeptide or the alteration of cellular morphology in situ. The specification does not teach the decreased level of expression of the HAAH polypeptide, or alterations in cell morphology in any CNS tissue, in vitro or in vivo (page 3, lines 8-16, of Paper No. 10).

Example 5 of the specification (page 47, line 26, to page 48, line 26) describes inhibition of AAH gene expression using FOCUS hepatocellular carcinoma cells. As noted by the Examiner, contacting the cells with AAH antisense nucleic acids led to reduced AAH expression and an improved phenotype, i.e., a more differentiated noncancerous "hepatocyte-like" phenotype. In addition, the treated cells were tested for growth in an art-recognized tumor model by inoculating the cells into nude mice. No tumors formed after inoculation of the AAH antisense-treated tumor cells into nude mice (page 48, lines 25-26, of the specification).

With respect to CNS tumor cells, Applicants have now tested AAH antisense nucleic acids with CNS and other tumor cell types for the ability to inhibit AAH gene expression and tumor cell growth (see accompanying Declaration of Jack R. Wands). Three AAH antisense nucleic acids with sequences complementary to sequences in exon 1 of the AAH gene were

tested. Antisense oligonucleotides (20 mers) were designed to bind to the 5' regulatory region of the AAH mRNA and to overlap with the 5' protein coding region (see attached Fig.1 of Declaration of Jack R. Wands). An AAH sense oligonucleotide was used as a negative control. All of the tested AAH antisense nucleic acids inhibited AAH gene expression in a CNS tumor cells (Sh-SySy neuroblastoma cells). In addition, the effect of the AAH antisense nucleic acids on tumor cell growth and migration was tested with a variety of tumor cell types: Sh-SySy neuroblastoma cells, 9L glioblastoma cells, H1 cholangiocarcinoma cells, NEC cholangiocarcinoma cells, RBE cholangiocarcinoma cells, and FOCUS hepatocellular carcinoma cells. The data indicated that AAH antisense nucleic acids inhibited tumor cell growth and migration of neuroblastoma, glioblastoma, hepatocellular carcinoma, and three different cholangiocarcinoma cell types.

Given the extensive guidance regarding antisense constructs and methods of administering them coupled with the working examples provided in the specification as filed and described in the accompanying Declaration of Jack R. Wands, Applicants submit that the application provides as adequate guidance for practicing the claimed invention.

Nature of the invention and State of the prior art

The fourth and fifth factors are not explained in Wands, but it might be reasonable to assume that the court was referring to the foundation in the art for the claims and the advance represented by the claims. The nature of the invention is a antisense therapy.

Antisense technology has been in development for nearly a decade. Successes with antisense therapy to specifically inhibit gene expression and reduce pathological symptoms in animals have been reported for several years (e.g., Bennett et al., 1997, J. Exp. Ther. 280:988-

1000, entitled "An ICAM-1 antisense oligonucleotide prevents and reverses dextran sulfate sodium-induced colitis in mice" (Attachment A); Mizuta et al., 1999, Nat. Biotech. 17:583-587, entitled "Antisense oligonucleotides directed against the viral RNA polymerase gen enhance survival of mice infected with influenza A" (Attachment B); and Driver et al., 1999, Nat. Biotech. 17:1184-1187, entitled "Oligonucleotide-based inhibition of embryonic gene expression" (Attachment C). A number of antisense compositions targeting a variety of genes are currently in human clinical trials (http://www.isispharmaceuticals.com/pipeline.htm; Attachment D) for treatment of cancers, diabetes, and inflammatory and infectious diseases. At least one antisense nucleic acid composition has been approved by the Food & Drug Administration (FDA) for human therapy. For example, an antisense compound targeting viral DNA for the treatment of CMV retinitis in humans was approved by the FDA for marketing in the United States in 1998 (http://www.isispharmaceuticals.com/products/cmvpage.htm; Attachment E). Thus, the foundation for making and administering antisense compositions for treatment of disease is well established.

Applicants have made a significant and valuable contribution to the field of cancer therapy by discovering, for the first time, that AAH antisense nucleic acids function to inhibit tumor cell growth. This discovery represents a major advance in the treatment of cancers, which are characterized by AAH overexpression.

Relative skill of those in the prior art

The invention spans the fields of molecular biology, antisense technology, and medicine. Applicants submit that the level of skill of those skilled in the art of molecular biology (e.g., the subfield of antisense technology) and medicine is very high. In each field, many years of post-

graduate training and experience is required to practice medicine or to conduct research regarding gene expression. Accordingly, armed with the information provided in the specification regarding identification and manufacture of AAH antisense nucleic acids and the guidance regarding methods of administering such compositions, those skilled in the art would readily be able to make and use the invention without undue experimentation.

Predictability or unpredictability of the art

At page 4, lines 1-5, and 10-14, the Examiner states:

> The published data indicates that only a small percentage of the antisense oligonucleotides which are tested in vitro are actually effective in the reduction of the target mRNA, and that the ability of the anti-sense oligonucleotides to bind to a target mRNA cannot be predicted due to the structure and conformation assumed by individual mRNA specie (Broaddus, et al., page122)
>
> Broaddus et al. teaches (sic) that a highly empirical approach to the testing of candidate anti-sense oligonucleotides is critical for the establishment of an antisense oligonucleotide as a therapeutic agent for the treatment of patients. This requirement has not been met by the instant specification, therefore, one of skill in the art would be forced into undue experimentation without reasonable expectation of success in order to practice the invention claimed.

The Broaddus quote to which the Examiner refers appears to reflect the effectiveness of antisense molecules tested before the rigorous *in vitro* evaluation suggested by the authors. The amended claims define specific AAH sequences to which the antisense nucleic acid are complementary, reflecting extensive research and testing by Applicants. In fact, the specification of the present application teaches precisely the type of *in vitro* testing (e.g., inhibition of gene expression and inhibition of cell growth) advocated by Broaddus. The test data presented in the specification and the Declaration of Jack R. Wands indicate that the antisense nucleic acids encompassed by the claims predictably inhibit gene expression and tumor cell growth. In fact, Broaddus reports that in his test system (targeting the myc-1 gene), the most effective antisense

oligonucleotide "was a sequence including the translation initiation codon, as has been described by other investigators." (page 127 of Broaddus). The AAH sequences encompassed by the claims are within exon 1 and are in close proximity or overlapping the AUG translation initiation codon.

Extensive pharmacokinetic/pharmacodynamic testing of antisense has now been carried out to determine the predictability of antisense drug clearance. Antisense nucleic acids were systemically administered; plasma clearance and accumulation in target tissues was monitored. Researchers have concluded that "[a]lthough antisense oligonucleotides represent a novel class of therapeutic agent, their activity may still be described in conventional pharmacologic terms." (Yu et al., 2001, J. Pharmacol. Therap. 296:388-395 at col. 1, p. 393; Attachment F). Thus, antisense drugs, such as those presently claimed, follow "a classic concentration-response relationship". (col. 1, p394, of Yu et al.)

Given the location and sequence of the claimed AAH antisense sequences, the high level of empirical testing described and carried out by Applicants, and the knowledge in the art regarding the pharmacockinetics/pharmacodynamics of antisense molecules *in vivo*, there is no reason to believe that the claimed methods would not predictably inhibit tumor cell growth as claimed.

Breadth of the claims

The pending claims require administration of a specific subset of AAH antisense nucleic acids defined by the portion of the AAH gene to which they are complementary. For example, the antisence nucleic acids correspond to sequences in defined regions of the AAH gene, e.g., 5'

AAH regulatory region, 5' AAH protein coding region, AAH sequence encoding a signal peptide, AAH sequences in exon 1 of the AAH gene, or sequences corresponding to the full-length naturally-occurring AAH cDNA. Moreover, dependent claims require specific tumor types to be treated; the tumor types recited by the claims have been shown to overexpress AAH. Inhibition of AAH expression in such tumor types has been shown to inhibit tumor growth.

In sum, the claims exclude inoperable embodiments, and the specification provides sufficient guidance to carry out the claimed methods. Therefore, Applicants submit that the claims are commensurate with the teachings provided in the specification.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance.

Applicants file concurrently herewith a petition for a two (3) month extension of time, together with a check for $445.00 to cover the fee pursuant to 37 C.F.R. § 1.17(a)(3). With the extension, this amendment is due on or before July 19, 2001. The Commissioner is hereby authorized to charge same, or credit any overpayment, to Deposit Account No. 50-0311 (Reference No. 21486-032).

Respectfully submitted,

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Appendix - Marked up version of amendments

In the claims:

Cancel claims 1-9, 11, 12, and 16-38.

10. (amended) A method of inhibiting tumor growth in a mammal comprising administering to said mammal a compound which inhibits expression of [HAAH,] alpha-ketoglutarate-dependent dioxygenase aspartyl (asparaginyl) beta-hydroxylase (AAH), wherein said compound is a AAH antisense nucleic acid comprising a sequence which is complementary to a 5' AAH regulatory sequence.

Add new claims 39-68.

--39. The method of claim 10, wherein said tumor is a glioblastoma.--

--40. The method of claim 10, wherein said tumor is a neuroblastoma.--

--41. The method of claim 10, wherein said tumor is a cholangiocarcinoma.--

--42. The method of claim 10, wherein said tumor is a hepatocellular carcinoma.--

--43. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a HAAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is complementary to a 5' portion of an AAH coding sequence.--

--44. The method of claim 43, wherein said tumor is derived from endodermal tissue.

--45. The method of claim 43, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.--

--46. The method of claim 43, wherein said tumor is a CNS tumor.--

--47. The method of claim 43, wherein said tumor is a glioblastoma.--

--48. The method of claim 43, wherein said tumor is a neuroblastoma.--

--49. The method of claim 43, wherein said tumor is a cholangiocarcinoma.--

--50. The method of claim 43, wherein said tumor is a hepatocellular carcinoma.--

--51. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a AAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is complementary to a AAH sequence encoding a signal peptide.--

--52. The method of claim 51, wherein said tumor is derived from endodermal tissue.

--53. The method of claim 51, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.--

--54. The method of claim 51, wherein said tumor is a CNS tumor.--

--55. The method of claim 51, wherein said tumor is a glioblastoma.--

--56. The method of claim 51, wherein said tumor is a neuroblastoma.--

--57. The method of claim 51, wherein said tumor is a cholangiocarcinoma.--

--58. The method of claim 51, wherein said tumor is a hepatocellular carcinoma.

--59. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a AAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is complementary to a AAH sequence in exon 1 of a AAH gene.--

--60. The method of claim 59, wherein said tumor is derived from endodermal tissue.

--61. The method of claim 59, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.--

--62. The method of claim 59, wherein said tumor is a CNS tumor.--

--63. The method of claim 59, wherein said tumor is a glioblastoma.--

--64. The method of claim 59, wherein said tumor is a neuroblastoma.--

--65. The method of claim 59, wherein said tumor is a cholangiocarcinoma.--

--66. The method of claim 59, wherein said tumor is a hepatocellular carcinoma.--

--67. The method of claim 59, wherein said nucleic acid comprises a sequence which is complementary to a full length naturally-occurring AAH transcript.--

--68. The method of claim 10, 43, 51, or 59, wherein said nucleic acid is a human AAH antisense nucleic acid.--